

verified spectrophotometrically. Nitric oxide was added from a stock solution, prepared under argon and purified across NaOH and a cold trap, by injection through a gas-tight Hamilton syringe with a Teflon seal. The concentration of nitric oxide stock solutions varied between 1.5 and 1.9 mM, as determined by a NO-specific electrode and oxidation of haemoglobin.

Titration of partially nitrosylated Hb with O₂. Oxygenation of Hb was achieved by sequentially injecting either 10 or 50 μ l of air into a 2-ml sealed vial with a gas-tight Hamilton syringe.

S-nitrosylation of Hb. Varying amounts of NO were added to deoxy Hb (200 μ M) for a final Hb concentration of 100–200 μ M (higher Hb concentrations pose technical problems in this experimental design) and vortexed under argon for a few seconds. Vials were then unsealed and vortexed in air until well oxygenated. The SNO-Hb yield was measured as described¹¹, with the exception that HgCl₂ was used at 6–7.5-fold the Hb concentration.

Titration of deoxy Hb with NO. 1 ml deoxy Hb (17.5 μ M) was prepared in a sealed quartz cuvette. NO was added sequentially from a stock solution by injection through a gas-tight Hamilton syringe with Teflon seal, and vortexed. Spectra were recorded after each addition. Difference spectra were calculated by subtracting the initial deoxy-Hb spectrum.

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Acetylcholine receptors containing the $\beta 2$ subunit are involved in the reinforcing properties of nicotine

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Release of the neurotransmitter dopamine in the mesolimbic system of the brain mediates the reinforcing properties of several drugs of abuse, including nicotine¹. Here we investigate the contribution of the high-affinity neuronal nicotinic acetylcholine receptor² to the effects of nicotine on the mesolimbic dopamine system in mice lacking the $\beta 2$ subunit of this receptor³. We found that nicotine stimulates dopamine release in the ventral striatum of wild-type mice but not in the ventral striatum of $\beta 2$ -mutant mice. Using patch-clamp recording, we show that mesencephalic dopaminergic neurons from mice without the $\beta 2$ subunit no longer respond to nicotine, and that self-administration of nicotine is attenuated in these mutant mice. Our results strongly support the idea that the $\beta 2$ -containing neuronal nicotinic acetylcholine receptor is involved in mediating the reinforcing properties of nicotine.

The mesolimbic dopaminergic system influences many important behaviours, such as locomotor activity and the reinforcing action of many drugs of abuse, including ethanol, cocaine and amphetamine¹. Both the locomotor-stimulating action and the reinforcing properties of nicotine are thought to be mediated through increasing dopamine release in the mesolimbic dopamine system². Nicotine administered systemically increases extracellular levels of dopamine in the dorsal and ventral (nucleus accumbens) striatum⁴ by acting on the nicotinic acetylcholine receptors (nAChRs) located on dopamine cell bodies and/or nerve terminals⁵. Accordingly, lesions of the mesolimbic dopaminergic neurons attenuate the locomotor-stimulating properties of nicotine⁶ and nicotine self-administration in rats⁷. The ten subunits of the neuronal nAChR identified so far combine to form a variety of receptor subtypes that are sensitive to nicotine, and at least six of these subunits ($\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 6$, $\beta 2$ and $\beta 3$) are expressed in mesencephalic dopaminergic neurons⁸. We therefore investigated which subtypes of the nAChR regulate dopamine levels in response to systemically administered nicotine and so contribute to the properties of nicotine that are likely to be involved in tobacco consumption.

We used 'knockout' mice that lacked the $\beta 2$ subunit of the nAChR³ in order to investigate nicotine-elicited dopamine release and related behaviours. We have already shown that these mutant mice have normal levels of the messenger RNAs encoding the $\alpha 2$ – $\alpha 7$ and $\beta 3$, $\beta 4$ subunits of the nAChR, but that they lack high-affinity binding of nicotine in the brain⁹. The mutant animals are indistinguishable from their wild-type siblings in a cage, but they lose their sensitivity to nicotine, as judged by their performance in a

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passive avoidance-learning task, and apparently acquire this behaviour more easily. In a first series of experiments, we used *in vivo* microdialysis to detect dopamine and its metabolites in $\beta 2$ -mutant mice and their wild-type siblings and to determine whether nicotine still elicits an increase in extracellular dopamine in the mutant animals. A 2-mm dialysis probe was used which spanned the ventral and part of the dorsal mouse striatum, and sampled both limbic and motor regions. Nicotine (free base) was administered intraperitoneally (i.p.) at 3 doses (0.030, 0.125 and 0.500 mg kg⁻¹) to anaesthetized mice and the dopamine concentration was measured in the dialysate for 120 min (Fig. 1). Nicotine doses were chosen after extensive testing of DBA/2 mice, C57/B16 mice and an F₁ cross

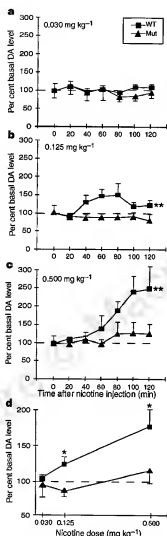


Figure 1 Nicotine-elicited dopamine (DA) release in $\beta 2$ -mutant mice and their wild-type siblings. **a–c**, Mice were injected i.p. with a solution of 0.030, 0.125 or 0.500 mg kg⁻¹ of nicotine (free base), and striatal extracellular DA levels were measured by microdialysis. Male mice aged 3–5 months were used. Results are reported as a percentage of the mean of three basal samples immediately before treatment. Basal dopamine levels in perfusate did not differ between the two groups; for mutant, 2.94 ± 0.38 nM; for wild type, 3.07 ± 0.49 nM. Means \pm s.e.m. are shown (n , 6–8 animals per group). Statistical analysis was carried out by a repeated-measures, two-way analysis of variance (ANOVA). ** $P < 0.01$, for wild-type versus mutant for both 0.125 and 0.500 mg kg⁻¹ doses. **d**, Dose-response curve of the effect of nicotine on extracellular dopamine levels in $\beta 2$ -mutant mice and their wild-type siblings after i.p. injection with 0.030, 0.125 or 0.500 mg kg⁻¹ nicotine. Average per cent changes compared to basal in the 6 samples collected after nicotine injection are shown. Statistics according to one-way ANOVA. * $P < 0.05$, mutant compared with wild type. WT, \pm mice; Mut, homozygous $\beta 2$ -mutant mice.

of these two strains, the strains included in the genetic background of the $\beta 2$ -mutant mice and their wild-type siblings³. The maximally effective dose of nicotine was 0.500 mg kg⁻¹ for dopamine release in C57B1/6 mice, whereas 0.030 mg kg⁻¹ of nicotine was ineffective in all strains. These doses are in the range used in studies of behavioural pharmacology and dopamine microdialysis in rodents^{4,11,12}. In $\beta 2$ ^{-/-} mice, 0.500 mg kg⁻¹ and 0.125 mg kg⁻¹ nicotine caused a peak increase of ~150 and 50%, respectively, in dopamine in the

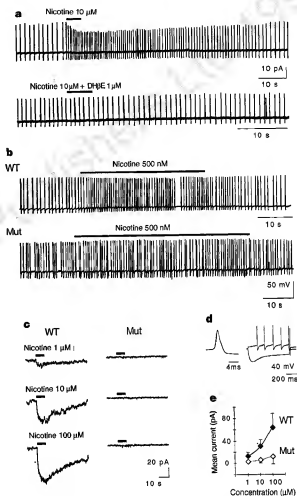


Figure 2 Patch-clamp recording of nicotine-evoked currents in substantia nigra (SN) pars compacta and ventral tegmental area (VTA) of wild-type and $\beta 2$ ^{-/-} mice. **a**, 10 μ M nicotine greatly increases the frequency of discharge of SN neurons recorded in the cell-attached configuration in neurons from wild-type (WT) mice. The nicotinic antagonist DH β E (1 μ M) reversibly blocks the action of nicotine in neurons from \pm animals. **b**, 500 nM nicotine moderately increases the firing rate of dopaminergic neurons from wild-type mice in the whole-cell configuration under current clamp conditions ($P < 0.001$). No increase in firing rate is seen in dopaminergic neurons from $\beta 2$ ^{-/-} mice. Statistical significance of the change in frequency in wild-type and mutant mice was tested with the matched *t*-test and the Wilcoxon test on the matched pairs of frequency value before and during nicotine application. **c**, Nicotine (1–100 μ M) elicits an inward current in SN and VTA neurons recorded in whole-cell configuration in wild-type but not in homozygous mutant mice. The slight increase in baseline noise after 100 μ M agonist application in mutant mice indicates that another type of nAChR may be present on these neurons. **d**, Broad spikes and characteristic response to hyperpolarizing/depolarizing current pulses of dopaminergic neurons. The left traces are a single action potential at expanded timescale; right trace corresponds to current injection (–250 pA, +150 pA) into the cell. Vertical scale is the same for the left and right traces. **e**, Dose-response curve for nicotine in wild-type and mutant mice. Values correspond to mean \pm s.d. of currents recorded at –70 mV. Each point corresponds to the average of 3 to 10 measures.

perfusate (Fig. 1a–d). In contrast to the results obtained with wild-type mice, dopamine levels were not significantly changed in the striatum of $\beta 2$ -mutant mice in response to any dose of nicotine (Fig. 1a–d). These results show that the $\beta 2$ subunit is a necessary component of the receptor(s) that are primarily responsible for the pharmacological effect of systemic nicotine on striatal dopamine release.

nAChRs located on mesencephalic dopaminergic cell bodies are necessary for dopamine release elicited by systemic nicotine administration¹⁵, and release of dopamine in the nucleus accumbens is likely to be involved in the reinforcing properties of nicotine⁶. We therefore investigated the effect of nicotine on the discharge frequency of dopaminergic neurons in thin slices of the substantia nigra (SN) and ventral tegmental area (VTA) in both cell-attached (Fig. 2a) and whole-cell (Fig. 2b) configurations to see whether there was a cellular response to nicotine in dopaminergic neurons from $\beta 2^{-/-}$ mice. Nicotine at 10 μ M increased the frequency of discharge of identified dopaminergic neurons (3/3 cells) and this effect was blocked by 1 μ M DH β E, a competitive nicotinic antagonist (Fig. 2a). We also tested the effect of 500 nM nicotine, which is close to the concentration of nicotine in the blood of human smokers (~500 nM nicotine from one puff¹⁴) and in rats injected i.p. with 0.1 mg kg⁻¹ nicotine¹⁵. In wild-type mice, 500 nM nicotine increased the discharge frequency in 10 out of 15 cells recorded (Fig. 2b). In contrast, no response was seen in 14 out of 15 dopaminergic neurons from $\beta 2^{-/-}$ mice (Fig. 2b). The average increase in discharge frequency following the application of 500 nM nicotine was 2.5 ± 0.5 (n = 15) in wild-type mice and 1.1 ± 0.3 (n = 15) in $\beta 2^{-/-}$ mice. This indicates that most, if not all, of the responses were due to the activation of $\beta 2$ -subunit-containing nAChRs. All cells recorded showed the characteristic broad spikes and response to hyperpolarizing and depolarizing current pulses characteristic of dopaminergic neurons¹⁶ (Fig. 2d). The lack of response to nicotine in these neurons from $\beta 2^{-/-}$ mice was confirmed in voltage-clamp experiments in which neurons in the SN and VTA of wild-type mice responded consistently to 1, 10 and 100 μ M nicotine with a small but detectable inward current (n = 46) (Fig. 2c, e) which was also blocked by 1 μ M DH β E (not shown). The agonist order of the response was compatible with that of $\alpha 4/\beta 2$ -containing nicotinic receptors *in vitro* (nicotine > cytisine) (results not shown)¹⁷. In 19 of 20 neurons tested from $\beta 2^{-/-}$ mice, the response of SN and VTA neurons to nicotine was abolished (Fig. 2c, e). Nicotine caused a small inward current in 1 of 20 neurons, however, and 100 μ M nicotine and cytosine could raise baseline noise in a few cells, indicating that nAChRs lacking the $\beta 2$ subunit, which have a much lower affinity for nicotine, persist in the mesencephalic neurons of the mutant mice. These results indicate that $\beta 2$ -subunit-containing receptors contribute to most, if not all, of the response to nicotine of mesencephalic dopaminergic neurons.

As nicotine also affects dopamine metabolism and reuptake¹⁸, we investigated whether mutant animals showed any alterations in these systems. Extracellular levels of homovanillic acid (HVA) and dihydroxyphenyl acetic acid (DOPAC), the principal products of dopamine metabolism, were measured by high-performance liquid chromatography (HPLC) following *in vivo* microdialysis, and were found not to be significantly changed in the ventral striatum of $\beta 2$ -mutant mice compared with their wild-type siblings (results not shown). We tested the levels of the dopamine transporter, the principal means of dopamine reuptake, by using quantitative autoradiography with tritiated WIN35,428, a cocaine analogue¹⁹, and found no difference in WIN35,428 binding in the ventral and dorsal striatum (mutant: 36.2 ± 2.2 fmol g⁻¹ tissue; WT: 39.3 ± 2.8 fmol g⁻¹ tissue) or ventral mesencephalon (mutant: 45.2 ± 3.1 fmol g⁻¹ tissue; WT: 45.7 ± 7.4 fmol g⁻¹ tissue) of $\beta 2$ -mutant mice compared with their wild-type siblings. However, the activity of the transporter may be modified in the mutant mice, despite the lack of a change in equilibrium binding measurements.

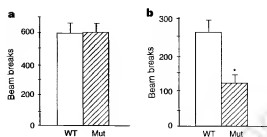


Figure 3 Locomotor activity of $\beta 2$ -mutant mice and their wild-type (+/+) siblings as measured in **a**, an unfamiliar environment, and **b**, in a familiar environment.

We also investigated whether mutant mice showed any difference in dopamine receptors and second messenger pathways involved in dopaminergic function. Quantitative receptor autoradiography using tritiated SCH23390 to detect D1 receptors²⁰ or tritiated raclopride to detect D2 receptors²¹, followed by computer-assisted image analysis, indicated that there was no significant difference in the levels of either D1 or D2 binding in ventral and dorsal striatum (D1: mutant, 410 ± 18 ; WT, 408 ± 14 ; D2: mutant, 87.3 ± 9.4 ; WT, 88.1 ± 6.3 fmol g⁻¹ tissue) or SN and VTA (D1: mutant, 312 ± 15 ; WT, 315 ± 10 fmol g⁻¹ tissue) in mutant mice and their wild-type siblings. Dopamine-stimulated cyclase assays²² did not reveal any significant changes in basal levels of cyclic AMP (mutant: 0.14 ± 0.03 nM; WT: 0.13 ± 0.03 nM) and cyclase activity (mutant: 16.8 ± 2.2 nM cAMP; WT: 17.4 ± 1.7 nM cAMP), forskolin-stimulated adenylyl cyclase activity (mutant: 305.7 ± 33.4 nM cAMP; WT: 387.2 ± 46.0 nM cAMP), or dopamine-stimulated adenylyl cyclase activity (mutant: 35.2 ± 5.0 nM cAMP, maximum WT: 38.1 ± 2.7 nM cAMP, maximum, nor was tyrosine hydroxylase activity²³ altered (mutant: 30.7 ± 3.0 c.p.m. per μ g protein; WT: 34.3 ± 3.5 c.p.m. per μ g protein). The dose-response of dopamine-stimulated adenylyl cyclase activity is available as Supplementary Information. The lack of change in dopamine receptor levels and in the related second messenger systems in $\beta 2$ -mutant mice confirms that regulation of the mesostriatal dopamine system by high-affinity nAChRs occurs primarily on dopaminergic neurons rather than at their target cells in the striatum²⁴.

The change in the regulation of dopamine release by nicotine in the $\beta 2$ -mutant mice suggested that endogenous acetylcholine acting through nAChRs might also act physiologically in regulating dopamine transmission. The modulation of locomotor activity is a primary behavioural output of the mesostriatal dopamine system. In rodents, dopamine denervation reduces both exploratory activity and habituated locomotion⁵. To determine whether the high-affinity nAChR could be involved in the control of spontaneous locomotor activity, we tested the exploratory behaviour and the habituated locomotor activity of mice lacking the $\beta 2$ -subunit of their wild-type siblings in standard locomotor activity cages. Whereas the exploratory activity of mutant and wild-type animals was similar (Fig. 3a), the locomotor activity of the $\beta 2^{-/-}$ mice after habituation decreased by ~50% (Fig. 3b) compared to the wild-type siblings, indicating that endogenous acetylcholine might act through high-affinity nAChRs in the physiological regulation of locomotion.

The mesolimbic dopamine system is thought to be a key substrate for nicotine self-administration²⁵; rodents will press a lever to receive intravenous infusion of nicotine²⁶, which results in activation of the terminal fields of the mesolimbic dopamine system²⁶. The role of the $\beta 2$ nicotinic receptor subunit in maintaining this operant response for nicotine was investigated in $\beta 2$ -mutant mice using an intravenous self-administration procedure^{27,28}. Mice were implanted with a jugular catheter and trained daily for cocaine

self-administration in operant chambers equipped with two nose-poke detectors, one active and one inactive. Once a stable baseline of cocaine responding in the active detector was reached, cocaine was replaced with nicotine at a very low dose, or with saline. As shown in Fig. 4, wild-type mice maintained significant specific operant response to nicotine during the 5 days following cocaine substitution. In wild-type mice, the pattern of nose-poke responses to nicotine, including some daily variations, resembled that in rats (ref. 28; and M. Epping-Jordan, personal communication). The performance of $\beta 2^{-/-}$ mice was significantly ($P < 0.05$) different from that of wild-type mice during the nicotine-substitution period, showing a decrease in nose-poke response (Fig. 4a; $P < 0.01$) and a clear-cut progressive reduction in discrimination between the active and inactive levers (Fig. 4b; $P < 0.01$). These effects resembled those of saline replacement in wild-type mice (Fig. 4), a substitution that does not maintain operant response. Thus, the ability of low doses of nicotine to maintain operant responding in mice trained to respond for cocaine in a substitution protocol was significantly attenuated in $\beta 2^{-/-}$ mice, suggesting that nicotine does not have a modulatory effect on the dopaminergic neurons of $\beta 2$ -mutant mice.

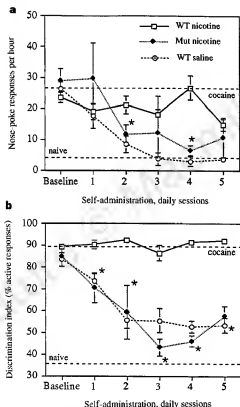


Figure 4 Intravenous self-administration of cocaine and nicotine in $\beta 2^{-/-}$ mice and their wild-type siblings with a history of cocaine self-administration. **a**, Number of active nose-poke responses per session. Dashed lines represent the average of the last 3 sessions of cocaine self-administration in all groups of mice ('cocaine'), or of spontaneous nose-poke behaviour in naive, sham-operated mice ('naive'). **b**, Per cent active responses; that is, the percentage of the number of active responses compared with the sum of active and inactive responses per session. Values of 50% indicate a lack of discrimination between active and inactive detectors. $\beta 2^{-/-}$ mice differed significantly from wild-type mice self-administering nicotine in either active nose-poke ($P < 0.05$) or discrimination index ($P < 0.01$), but did not differ from wild-type mice during saline-induced extinction (ANOVA). The increase in the variability of nose-poke response of $\beta 2^{-/-}$ mice on the first day of nicotine treatment was due to increased responding of some mice, usually interpreted as a transient overresponse to 'reinforcer' devaluation. Asterisks represent post-hoc comparisons of the wild-type nicotine group and the $\beta 2^{-/-}$ group (* $P < 0.05$).

These results on nicotine-elicited dopamine release and the electrophysiological response of dopaminergic neurons to nicotine indicate that a $\beta 2$ -subunit-containing nAChR contributes to the effects of nicotine on the dopamine mesostriatal system. The changes in habituated locomotor activity of mutant mice in the absence of administered nicotine imply that the nicotinic regulation of the dopamine system may have consequences on the endogenous regulation of locomotion. Also, the changes in the self-administration of nicotine by $\beta 2$ -mutant mice indicate that a $\beta 2$ -subunit-containing nAChR is involved in mediating the reinforcing properties of nicotine, as determined by the attenuated response of $\beta 2$ -mutant mice when nicotine was substituted for cocaine in the self-administration protocol.

Experiments on nicotine-enhanced glutamatergic and cholinergic transmission in the developing chick brain using an $\alpha 7$ -type nAChR²⁹ indicate that low concentrations of nicotine can activate several different subtypes of nAChR, some of which do not contain a $\beta 2$ subunit. Many systems may converge to mediate the reinforcing properties of nicotine. Taking into account this heterogeneity, our results provide evidence that the lack of nicotine-mediated stimulation of the mesolimbic dopaminergic system is associated with attenuation of nicotine self-administration in $\beta 2$ -subunit-mutant mice. These data, combined with other evidence³⁰ indicate that an increase in dopamine release mediated through $\beta 2$ -containing nAChRs is one of the primary substrates for the addictive properties of nicotine.

Methods

Microdialysis. Microdialysis and dopamine measurement were adapted from ref. 30. On the day of the experiment, mice were anaesthetized with a mixture of air and halothane (1.4% of halothane in the air flow of 1.51 min⁻¹) and placed in a stereotaxic frame. The skull was exposed and one hole was drilled to allow implantation of the microdialysis probe (CMA 11, o.d. 0.24 mm; 2-mm cuprofen dialysing membrane). The probe was implanted into the ventral striatum ($A = 0$, $L = 2.0$, $V = 4.5$) and was perfused at a flow rate of 2 μ l min⁻¹ with Ringer's solution. The site of probe implantation was chosen after extensive testing of rostral and caudal sites in ventral striatum ($n = 5$ –6 animals per location), including core ($A = 0.7$, $L = 1.6$, $V = 4.8$) and shell ($A = 1.2$, $L = 1.0$, $V = 5.0$) of the nucleus accumbens. This location showed the most reliable and marked increase in nicotine-elicited dopamine release in our preparation. Collection of perfusate samples began 120 min after probe implantation, with 40- μ l samples being collected every 20 min. Nicotine was freshly dissolved in Ringer's solution and injected i.p. after collecting three stable samples that constituted the baseline. Upon completion of the experiment, mice were killed by an overdose of halothane and their brains were sectioned to verify the probe location. Dopamine, DOPAC and HVA concentrations were measured by HPLC with electrochemical detection. Limit of detection for dopamine was 2 fmol per sample.

Patch-clamp recording. Coronal slices through SN and VTA were obtained from 10–15-day-old mice for the nicotine dose-response curve and 1–2-month-old mice for experiments on discharge frequency. Patch-clamp recording was done as described³. To improve the quality of the slices, a step of intracardiac superfusion of the mice with an ice-cold sucrose solution was added before killing the animals at 1–2 months of age. The sucrose solution contained (in mM): sucrose 283, NaHCO₃ 26, KCl 1.3, CaCl₂ 2, MgCl₂ 2, D-glucose 10, and was bubbled with 95% O₂, 5% CO₂ (pH = 7.2). For blocking experiments, dihydropyridine (DHP) was applied first in the bath and then together with nicotine.

Locomotor activity. A clear plastic cage 25.4 cm high \times 45 cm long and 25.4 cm wide was used for all locomotor activity experiments. Movement was detected by eight infrared photobeam detectors and transducers set 1.5 cm above the floor of the apparatus and measured by a PC. Mice of 6–7 months old that were naive to the apparatus were used in novelty testing; activity was measured for 15 min, with time points being taken every 5 min ($n = 14$ per group). For locomotion in a familiar environment, activity was measured after repeated exposure to the apparatus for 60 min at intervals of 20 min ($n = 10$ per group). For all experiments, each mutant mouse was compared with a wild-

type (+/-) sibling of the same sex from the same litter. Locomotion experiments were run by an observer blind to the genotype of the animals being tested. Tests were run from 10.0 a.m. to 2 p.m. Data are presented as mean \pm s.e.m. Statistical analysis was performed using ANOVA ($P < 0.05$ mutant compared with wild type).

Self-administration. Adult male mice (wild-type and mutant siblings of parents backcrossed 3 generations to C57 B/16 inbred mice) were implanted with a silastic catheter in the jugular vein under halothane anaesthesia and tested in opaque cages equipped with two nose-poke detectors, one active, the other inactive (V. Deroche et al., manuscript in preparation). All mice were first trained with cocaine (0.8 mg kg⁻¹ per injection, delivered in 50 μ l per 2 s, with 20-s time-out period) under fixed-ratio (FR) 1 for 2–4 sessions, then under FR2 until stable baseline was reached. Spontaneous nose-poke behaviour, measured in naive, sham-operated mice when both detectors were inactive, was low and non-discriminatory (2.6 ± 0.6 per h), and did not differ between $\beta 2^{-/-}$ mice and wild-type mice (not shown). The baseline for each mouse was defined as 3 consecutive sessions with less than 30% deviation from the mean and at least 75% active-specific response. During 5 consecutive daily sessions, cocaine was replaced with nicotine (0.03 mg kg⁻¹ per injection, delivered in 50 μ l per 2 s, 20-s time-out period, under FR 2 schedule) in wild-type mice ($n = 5$) and $\beta 2^{-/-}$ mice ($n = 5$). In a second group of wild-type mice ($n = 5$), cocaine was replaced with saline, forcing the operant responding to extinction. Cocaine and nicotine bitartrate (Sigma) were freshly dissolved in saline before each experiment.

Equilibrium binding. Receptor autoradiography was done as described^{19–21}. Briefly, following 30 min preincubation in the appropriate buffer, 14- μ m coronal brain sections from wild-type and $\beta 2$ -mutant mice were incubated at room temperature for 120 min with 5 nM [³H]WIN35,428 (84.5 Ci mmol⁻¹), NEN²² or for 60 min with either 1.5 nM SCH23390 (70 Ci mmol⁻¹), NEN²³ or 3 nM raclopride (82.4 Ci mmol⁻¹), NEN²⁴. Slides were washed in ice-cold buffer twice for 1 min (WIN35,428) or for 5 min (SCH23390 or raclopride), and exposed to hyperfilm together with appropriate standards [³H]microscale; Amersham). Nonspecific labelling was determined in the presence of 30 μ M cocaine for the dopamine transporter and 1 μ M (+)-butaclamol for D1 and D2. Siblings of the same sex and litter were used for all experiments ($n = 4–6$ per group).

Cyclase and tyrosine hydroxylase assays. Dopamine-stimulated cyclase activity was measured in striatal homogenates from wild-type and $\beta 2$ -mutant siblings of the same sex and same litter by following published protocols²⁵ and using the Amersham Biotrack scintillation proximity assay to determine cAMP levels. For each animal ($n = 6$ per group), the striatum from one side of the brain was used for dopamine-stimulated cyclase assays, and the striatum from the other side of the brain was used to measure tyrosine hydroxylase activity as described²⁶. Briefly, after homogenization, samples were incubated with [³H]tyrosine and tetrahydrobiopterin. End products were separated from unreacted [³H]tyrosine by treatment with activated charcoal. Results are reported as the number of counts incorporated minus background per μ g protein assay.

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Extreme Th1 bias of invariant V $\alpha 24$ J α Q T cells in type 1 diabetes

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Type 1 diabetes (insulin-dependent diabetes mellitus, IDDM) is a disease controlled by the major histocompatibility complex (MHC) which results from T-cell-mediated destruction of pancreatic β -cells¹. The incomplete concordance in identical twins and the presence of autoreactive T cells and autoantibodies in individuals who do not develop diabetes suggest that other